**HYPOLIPIDEMIC AND ANTIOXIDANT CAPACITY OF METHANOL LEAF EXTRACT OF KIGELIA AFRICANA IN ALLOXAN INDUCED DIABETIC ALBINO RATS.**

**BY**

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**U14/NAS /BCH/028**

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**GODFREY OKOYE UNIVERSITY, UGWUOMU-NIKE, ENUGU.**

**JULY, 2018**

**TITLE PAGE**

**HYPOLIPIDEMIC AND ANTIOXIDANT POTENTIALS OF METHANOL LEAF EXTRACT OF KIGELIA AFRICANA IN ALLOXAN INDUCED DIABETIC ALBINO RATS**

**BY**

**OKWUAYA NNEOMA STEPHANIE**

**U14/NAS/BCH/028**

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**SUPERVISOR**

**DR. UHUO, EMMANUEL.N**

**JULY, 2018**

**CERTIFICATION PAGE**

This is to certify that this work titled Hypolipidemic and antioxidant capacity of methanol leaf extract of kigelia Africana on alloxan induced diabetic albino rats is the original work of Okwuaya, Nneoma Stephanie with the registration number U14/NAS/ BCH/028.

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**DEDICATION**

I dedicate this work to the Almighty God who is the giver of life , good health for his grace and mercies all through my stay in Godfrey Okoye University , I am forever grateful .

**ACKNOWLEDGEMENT**

First of all, I give sincere thanks with a sense of humility to my supervisor Dr. Uhuo Emmanuel who made it possible for this work to be a success, he is indeed a legend ad I say a very big thank you him. And to the Dean Of Natural and Applied Sciences Prof. Chidi Ughegbu, I really appreciate every single time you have been of help to me. I also want to appreciate the head of department chemical sciences for his effort in ensuring orderliness in the department. To the chemical science department of Godfrey Okoye University, the secretary and the lecturers especially Mr obiudu keneth , Mr frank , Mr engwa ,Miss Amanda etc who in one way or the other has enlightened me in my academic pursuit. They have really done well and I am very grateful to them. Thank you so much my tutors.

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**ABSTRACT**

Diabetic mellitus is a metabolic disorder resulting from a defect in insulin secretion , insulin action or both . The deficiency of insulin leads to chronic hyperglycemia with disturbances of carbohydrate , fats and protein metabolism*. Kigelia Africana* is a sausage tree known to have medicinal values. The aim of this work is to evaluate the hypolipidemic and antioxidant capacity of the plant , methanol leaf extract of *kigelia Africana* which was used for the study. The leaves were ground into a powdered form , weighed and soaked with 500ml of methanol for 72hours. It was filtered and the filtrate was concentrated using rotary evaporator at 30 degrees centigrade. Alloxan was induced into 15 male wister albino rats and 5 male wister albino rats were used for the normal control group. They were grouped into four groups : group 1 which was the normal contol group , group 2 was the diabetic rats not treated , group 3 the diabetic rats treated with the standard drug (Glibenclamide) , group 4 diabetic rats treated with 500mg/kg body weight of the extract of kigelia Africana orally for 14 days. The rats were bled and their blood sample were collected and assayed for the biochemical parameters. The result showed that there was a significant decrease (p<0.05) inTAG for the group four which received the plant extract compared to the normal in group one. For total protein it showed a significant decrease (p<0.05) in the total protein level of the treated groups compared to the normal.The results obtained in GPX study when compared to the normal control showed a significant decrease (p<0.05) in the group four treated with the extract.An increase (p<0.05) not too significant was observed in the test group 4 MDA treated with the plant extract compared with other groups.

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**CHAPTER ONE**

**INTRODUCTION**

**1.1 BACKGROUND OF STUDY**

Diabetes mellitus is a metabolic disorder resulting from a defect in insulin secretion, insulin action or both. Insulin deficiency in turn leads to chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism (Kumar *et al.,* 2011). During diabetes, failure of insulin-stimulated glucose uptake by fat and muscle cause glucose concentration in the blood to remain high, consequently glucose uptake by insulin independent tissue increases. Increased glucose flux both enhances oxidant production and impairs antioxidant defenses by multiple interacting non-enzymatic, enzymatic and mitochondrial pathways. This hyperglycaemia-induced oxidative stress ultimately results in modification of intracellular proteins resulting in an altered function and DNA damage, activation of the cellular transcription (NFK B), causing abnormal changes in gene expression, decreased production of nitric oxide, and increased expression of cytokines, growth factors and pro-coagulant and pro-inflammatory molecules.

The plant *kigelia Africana*has many medicinal properties due to the presence of numerous secondary metabolites. These compounds include iridiods, flavonoids, naphthoquinones and volatile constituents (Houghton,2002). Experimentally, the plant has shown antibacterial, antifungal, antineoplastic, analgesic, anti-inflammatory and antioxidant properties (Saini *et a*l., 2009). Crude extracts of herbs and spices and other materials rich in phenolics are of increasing interest in the food industry because they retard oxidative degradation of lipids and thereby improving the quality and nutritional value of food. Flavonoids, are groups of polyphenoli compounds with known properties, which include free radical-scavenging and antinflammatory activities.

**1.2 STATEMENT OF THE PROBLEM**

Improvement has occurred in global health status in the past century which is now a cause for celebration. Therefore, public health professionals can feel proud of their contribution to these achievement even as they appreciate the complexity of the underlying driving force, many of which lie outside tradition public health work.but this satisfaction must be tempered by emerging concerns against the recent evidence suggesting that based current trends many low income countries are unlikely to achieve desired health target by 2015 due to devastating disease and overwhelming failing health system.

The literature review survey revealed that there is no experiment evidence of antidiabetic and hypolipidemic effect of the plant . Therefore the present work was undertaken to explore the antidiabetic and hypolipidemic potential of *kigelia Africana* methanol leaf extract of the plant in alloxan induced diabetic rats.

**1.3 AIM OF THE STUDY**

The research is aimed at investigating the hypolipidemic and antioxidant potential of methanol leaf extract of *kigelia Africana* in alloxan induced diabetic rats.

**1.4 OBJECTIVES OF THE STUDY**

Specifically to

1. Determine the effect of *kigelia Africana* methanol leaf extract on antioxidant enzymes.

2. Determine the effect of *kigelia Africana* methanol leaf extract onMDA of diabetic rats.

3. Determine the effect of *kigelia Africana* methanol leaf extract on oxidative parameters of alloxan induced diabetic rats.

**CHAPTER TWO**

**LITERATURE REVIEW**

**2.1 GENERAL INFORMATION**

*Kigelia Africana* or commonly known as the sausage tree is known to be useful in treating stomach problem, pneumonia, toothache, increases production of milk in the lactating woman, treating sores, skin ulcer and many more.

*K.Africana* (lam) benth k.pinnata belongs to the family of bignoniaceae.The name *‘Kigelia’* is a native African name.It’s a highly variable monospecific genus of the family Bignoniaceae. Its other names are Worsboom (Afrikaans), Modukghulu, Pidiso (North Sotho), umVongotsi (Siswati); Mpfungurhu (Tsonga), Muvevha (Venda) Sausage tree (English) Pandoro (west nigeria)

First off, sausage tree’s fruits can be used in a traditional beer enjoyed by the Kikuyu people. In order to make the fruits safe for beer brewing, they must first be sun-dried, then fermented with sugar cane juice for around 24 hours, then dried again, before finally being added to a large barrel of sugar cane juice, where they’ll ferment for around four days. The final beverage should taste sour, smell boozy, and hopefully not act as an immediate emetic.

Infections of the genito-urinary tract, particularly venereal diseases, are treated both internally and externally with preparations of the roots, bark, leaves, stems and twigs. In West and Central Africa, palm wine, in which dried and ground bark is macerated, is taken against syphilis. Venereal diseases in children are treated simultaneously with a drink and wash prepared from decocted bark. A commercial product containing *Kigelia africana* stem bark is used to treat Candida albicans infections. In Côte d'Ivoire, renal and bladder ailments are treated with medicaments containing the bark and leaves of *Kigelia africana* and several other medicinal plants.

**2.2 ORIGIN AND GEOGRAPHICAL DISTRIBUTION**

*Kigelia africana* occurs throughout tropical Africa, particularly in the drier regions. It is also found in South Africa (Northern Provinces, Kwazulu-Natal) and Swaziland, but does not occur in Mauritania, São Tomé and Principe, or the Indian Ocean islands. It has been introduced as an ornamental to Cape Verde and Madagascar, as well as to Iraq, Pakistan, India, China, South-East Asia, Australia, Hawaii and Central and South America.

*Kigelia* is a genus of flowering plants in the family Bignoniaceae. The genus comprises only one species, *Kigelia africana*, which occurs throughout tropical Africa from Eritrea and Chad south to northern South Africa, and west to Senegal and Namibia. The *Kigelia* grows a fruit that is up to 2 feet long, weighs about 15 lbs, and looks like sausage.

*Kigelia africana* is used in both traditional and orthodox medicines to treat malignant neoplasms such as skin melanoma, tumours and breast cancer. Traditional preparations include extracts, poultices and powders of the bark or fruits; topical creams containing extracts of the fruits are produced commercially. They are huge and greyish brown, 800 x 120 mm. hangs from long stalks, from December (summer) to June (winter) and weighs anything up to 9 kg!

These weird looking fruits are highly poisonous to humans. But baboons, monkeys, and bush pigs eat them happily. The flowers eaten by antelope and pollinated by bats.

**2.3 SCIENTIFIC CLASSIFICATION**

Binomial name*: kigelia africana*

Kingdom: Plantea

Class: angiosperms

Order: lamiales

Family: Bignoniaceae

Tribe: coleeae

Genus: kigelia

Species: k.africana

Igbo name : ogbeala

(Lam.) Benth.

****

**2.3.1 : Leaf of *Kigelia Africana*** (Eliud, 2018)

**2.4 MEDICAL BENEFITS OF *KIGELIA AFRICANA***

1. The medicinal uses of the sausage tree are rather sexual in nature. The fruit can be ground up and mixed with water to help young men improve their manhood. It’s also rubbed on male genitals or on women’s breasts to make them larger.

2. An infusion can be made from the ground bark and fruits to treat stomach problems in children, and an infusion of the roots and bark can be taken to treat pneumonia.

3. A decoction made from the bark can be gargled to relieve a toothache.

4. An infusion of the bark can be used to wash the head as a way of treating epilepsy.

5. Due to its strong antimicrobial properties, including antibacterial, antifungal and antiviral, extracts of leave and barks have become popular in cosmetics .

6. It can also be used for treating sores, skin ulcer and cancer.

7. The seeds of ripe fruits can also be roasted in warm ash and consumed and are reported to be energy-rich, with significant amounts of phosphorous, protein, and lipids. In turn, the seed oil is rich in oleic acid and essential fatty acids and has the potential to be an important nutritional resource.

8. The leaves of the *K.Pinnata* are consumed by lactating women in various parts of sub-Saharan Africa as they are thought to enhance the volume and quality of breast milk.

9. The dried leaves contain levels of essential amino acids can provide beneficial health benefits as well as other minerals and nutrients including calcium, magnesium, and iron.

10. It is reported that the Tonga women of the Zambezi Valley regularly apply cosmetic preparations of the fruits to their faces to maintain a blemish-free complexion.

**2.4.1 Antibacterial and Antifungal**

A biologically monitored fractionation of the methanolic extracts of the root and fruits led to the isolation of the naphthoquinones, kigelinone, iso-pinnatal, dehydro-α- Lapachone, and lapachol and the phenyl propanoids, p-coumaric acid and ferulic acid as the compounds responsible for the observed antibacterial and antifugal activity (Binutu *et al*., 1996). The compounds isolated were tested for their activities against Staphylococcus aureus, Bacillus Subtilis, Corynebacterium diphetheriae, Aspergillus niger, A. flavus, Candida albicans and Pullularia pullularis (Aureobasiduim sp). The steroids and flavonids are hygroscopic and have fungicidal properties. Chemical investigation showed that the aqueous extracts of the stem bark of the plant contain irridoids as major components. In the light of traditional uses of this plant, antimicrobial activities of the aqueous extracts and two major irridoids were tested against Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus and Candida albican. The crude aqueous extracts showed significant antimicrobial activity, which could be partially explained by the activity of the irridoids present (Akunyili *et al*., 1991). The fruits are popular sources of traditional medicine throughout Africa. The stem bark has been widely analyzed for pharmacological activity but fruit is limited despite more extensive use in traditional remedies.

In the microtitre plate bioassay, stem bark and fruit extracts of *K.africana* showed similar antibacterial activity against Gram negative and Gram positive bacteria. A mixture of free fatty acids exhibiting antibacterial effect was isolated from the ethyl acetate extract of the fruits using bioassay-guided fractionation. Palmitic acid, already known to possess antibacterial activity, was the major compound in this mixture. These results confirm antibacterial activity of *K.africana* fruits and stem bark, and support the traditional use of the plant in therapy of bacterial infections (Grace *et a*l., 2002). A disc diffusion susceptibility test was used to screen concentrated extracts from the bark of 3 medicinal plants (Aistonia boonei de wild, Morinda lucida Benth and *K. africana*) for antimicrobial activity Solvents with different polarities were used for the extraction (methylene Chloride, ethyl acetate, 95% ethanol and acetonitrite), and the extracts were tested against Candida albicans, Staphylococcus aureus, Enterococcus faecalis, Escherichia coli and Pseudomonas aeruginosa. The patterns of inhibition varied with the plant extract, the solvent used for extraction and the organism tested. The largest zone of inhibition was observed for ethanol extracts of *K. africana* against S. aureus and P. aeruginosa. S. aureus was the most inhibited new organism. No inhibitory effects were observed against C. albican. The extent of the inhibition of the bacteria was related to the concentration of the plant extract (Kwo and Craker, 1996).

**2.4.2 ALTERNATIVE USES OF KIGELIA AFRICANA**

*Kigelia africana* is widely used throughout Africa for a variety of purposes, particularly in local medicine, and more recently in commercial applications to treat various skin complaints.

The diversity of complaints against which the plant is used includes fainting, anaemia, sickle-cell anaemia, epilepsy, respiratory ailments, hepatic and cardiac disorders, and nutritional illnesses such as kwashiorkor, rickets, wasting and weakness. The leaves are sometimes used to prepare a general tonic for improved health and growth. Aqueous fruit preparations are applied as a wash or rub to promote weight gain in infants.

The roots, bark, leaves, stems, twigs and fruits are used to treat digestive disorders. Administration is typically by oral ingestion or as an enema. The roots, bark and ripe or unripe fruits are taken as a laxative or emetic, to treat chronic and acute digestive disorders and against gastric infections. Remedies containing the fruits of *Kigelia africana* and Capsicum or Anthocleista are taken internally to relieve constipation or haemorrhoids.

Infections of the genito-urinary tract, particularly venereal diseases, are treated both internally and externally with preparations of the roots, bark, leaves, stems and twigs. In West and Central Africa, palm wine, in which dried and ground bark is macerated, is taken against syphilis. Venereal diseases in children are treated simultaneously with a drink and wash prepared from decocted bark. A commercial product containing*Kigelia africana* stem bark is used to treat Candida albicans infections. In Côte d'Ivoire, renal and bladder ailments are treated with medicaments containing the bark and leaves of *Kigelia africana* and several other medicinal plants.

*Kigelia africana* is widely used to treat gynaecological disorders. Aqueous preparations of the roots, fruits and flowers are administered orally or as a vaginal pessary. The fruits and bark are used to promote breast development in young women, or in contrast to reduce swelling and mastitis of the breasts. The fruits are further employed as a galactogogue. The bark and leaves are decocted and administered as an abortifacient.

Sexual complaints such as infertility, poor libido, sexual asthenia and impotence are treated with medicines containing the fruits, roots or leaves. A small amount of unripe fruit is chewed, or an aqueous preparation is taken orally as a sexual stimulant, and the intoxicating traditional beer to which they are added is drunk as an aphrodisiac. Excessive use of *Kigelia africana* to treat male sexual complaints is said to induce scrotal elephantiasis, although in some regions the fruits are used to remedy this condition.

Powders and infusions of the bark, leaves, stems, twigs or fruits are used to clean and dress flesh wounds and open sores. Many dressings, topical treatments and infusions containing *Kigelia africana* are also used for their analgesic and anti-inflammatory properties. The bark, stems, twigs, leaves and fruits are infused and taken orally, or applied locally, to relieve rheumatism, sprains, haematoma and bruising; a decoction of the fruit and bark is used to relieve toothache and headache. Snake bite antidotes are made with an infusion of the fruits, stems, leaves, twigs or bark, taken orally or rubbed onto the bite. A fruit decoction is used to treat oedema of the legs.

*Kigelia africana* is used to treat infectious diseases including leprosy, impetigo, and worm infestations in the blood. Dermal complaints and infections, such as whitlows, cysts, acne and boils, are treated with traditional medicines containing the fruits, and less frequently, the bark. Sore eyes are treated with drops made from flower sap mixed with water. Commercially manufactured products are used for symptomatic relief or cure of skin conditions including, among others, sunburn, chafing, psoriasis, itchy scalp and nappy rash. A broad-spectrum antimicrobial cream, reputedly effective against a number of common microbial infections, is produced from the stem bark. Fungal infestations such as ringworm, mycosia and athlete's foot are washed with the water in which bark has been macerated, and preparations containing the leaves and fruits applied locally. A root decoction is administered against internal parasitic infestations, notably tapeworm.

*Kigelia africana* is used in both traditional and orthodox medicines to treat malignant neoplasms such as skin melanoma, tumours and breast cancer. Traditional preparations include extracts, poultices and powders of the bark or fruits; topical creams containing extracts of the fruits are produced commercially.

The fruits, and sometimes other plant parts, are also much used in ethnoveterinary medicine to treat digestive system disorders, leg oedemas, dermal irritations and infections, mastitis and retained placenta. Brucellosis and Newcastle disease are also treated with Kigelia africana.

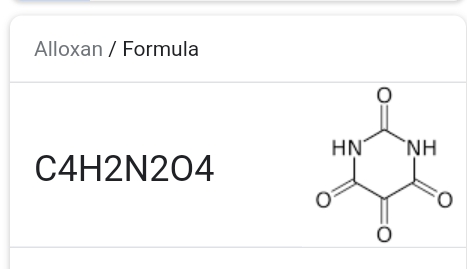
Due to the unusual fruits and large flowers, Kigelia africana is considered a striking ornamental plant, and the fruits are used as florists' material. The thick stem is an attractive feature for bonsai. The tree is sometimes planted as a boundary marker, but usually at roadsides and for shade. Due to its occurrence along watercourses, it is suitable for erosion control and riverbank stabilization.

**2.4.3 CHEMICAL CONSTITUENTS OF *KIGELIA AFRICANA***

*K. africana* plant has many medicinal properties due to the presence of numerous secondary metabolites. These compounds include irridiods, flavonoids and naphthoquinones and volatile constituents (Houghton, 2002;). Pinnatal and isopinatal were isolated from tropical trees that belong to the plant family of Bignoniaceae. Pinnatal was found in a root bark extract of the plant. Thin layer chromatography (TLC) examination of the most active fractions of both stem bark and fruits showed the presence of some major components which were found to be norviburtinal and Bsitosterol. isolated a furanone derivative, 3- (21-hydroxyethyl)-5-(2”- hydroxypropyl)- dihydrofuran -2(3H)- one and four irridoids, 7 hydroxy viteoid II, 7 hydroxy eucommic acid, 1- hydroxyl – 10- deoxyecuommiol and 10-deoxy eucommoil together with seven known irridoids, jiofuran, jioglutolide, 1-dehydroxy-3, 4- dehydroaucubigenin, des-p- hydroxybenzoyl kisasagenol B, ajugol, verminoside and 6-trans-caffeoyl ajugol from the fruit. They also isolated a phenyl propanoid derivatives identified as 6-p- coumaroyl-sucrose together with ten known phenylpropanoid and phenylethanoid derivativeand a flavonoid glycoside from fruits of *K.africana*. The structures of the isolated compounds were characterized by different spectroscopic methods. Isolated *kigelia* as the major constituent of the plant from the root heartwood.

**2.5 ALLOXAN**

Alloxan and streptozotocin are the most prominent diabetogenic chemicals in diabetes research. Both are cytotoxic glucose analogues. Although their cytotoxicity is achieved via different pathways, their mechanisms of beta cell selective action are identical (Lenzen,2007). In 1938 Wohler and Liebig synthesized a pyrmidine derivative, which they later called alloxan (Lenzen *etal*.,1996). In 1943, interest in alloxan increased when Dunn and Mc letchie reported that it could induce diabetes in animals as a result of the specific necrosis of the pancreatic beta cells. The resulting insulinopenia causes a state of experimental diabetes mellitus called alloxan diabetes. The reduction product of alloxan, dialuric acid, has been shown to be diabetogenic in animals and to cause ultrastructural changes identical to those observed in response to alloxan . It was reported that streptozotocin is diabetogenic and could cause diabetes by specific necrosis of the pancreatic beta cell. Research has provided a unifying explanation for selective toxicity of these most prominent diabetogenic agents.

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**C4H2N2O4**

**2.5.1 : chemical structure of Alloxan.**

**Source from: The Merck Index, 2018.**

**2.5.2 MECHANISM OF ACTION**Alloxan has two distinct pathological effects: it selectively inhibits glucose-induced insulin secretion through specific inhibition of glucokinase, the glucose sensor of the beta cell, and causes a state of insulin-dependent diabetes through its ability to induce ROS formation, resulting in the selective necrosis of beta cells (Lenzen, 2008). Due to its chemical properties, in particular the greater stability ,streptozotocin is the agent of choice for reproducible induction of a diabetic metabolic state in experimental animals (Lenzen *et al.,*1996). Alloxan on the other hand, as a model compound of ROS-mediated beta cell toxicity, is the agent with the greater impact upon the understanding of ROS mediated mechanisms of beta cell death in type 1 and type 2 diabetes mellitus.

**2.5.3 CHEMICALPROPERTIES OF ALLOXAN**

Chemical name---2,4,5,6-tetraoxypyrimidine

2,4,5,6-pyrimidinetetrone

Chemical structure--- oxygenated pyrimidine derivatives

Chemical properties---very hydrophilic , beta cells toxic glucose analogue (partition coeffient – 1.8)

Mode of toxicity ---ROS

Chemical reactivity---thiol reagent that is reduced by dialuric acid in the prescence of GSH and other thiol.

**2.5.4 IMPACT OF ALLOXAN UPON BETA CELLS**

Because it selectively kills the insulin producing beta-cells found in the pancrease , Alloxan is used to induce diabetes in laboratory animals . This occurs most likely because of the selective uptake of the compound due to its structural similarity to glucose as well as the beta-cells is highly efficient in the uptake mechanism(GLUT2)

Some studies have shown that alloxan is not toxic to the human beta-cells , even in high doses , probably because of differing glucose uptake mechanism in human and rodents.Alloxan exert its diabetogenic action when it is administered intravenously, intraperitorically or subcutaneously .The dose of alloxan required the animal species , route of administration and nutritional status . Fasted animals are most susceptible to Alloxan .

**2.6 DIABETES MELLITUS (DM)**

According to WHO (1999), the term diabetes mellitus describes a metabolic disorder of multiple aetiology characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both. (Samreen, 2009) also defined diabetes mellitus as a disorder that affects the body’s ability to make or use insulin. Insulin is a hormone produced in the process that helps transports glucose (blood sugar) from the blood stream into the cells so that they can break it down and use it for fuel. Therefore, people cannot live without insulin. Diabetes results in abnormal levels of glucose in the bloodstream. This can cause several short term and long term consequences ranging from brain damage, amputation and heart disease (Samreen, 2009). The long term effects may be coupled with dysfunction and failure of various organs. DM may present characteristic symptomssuch as thirst (polydipsia), polyurial (frequent urination), blurring of vision and weight loss. In its most severe forms, ketoacidiosis or a non-ketotic hyperosmolar state may develop and lead to stupor, coma and, in absence of effective treatment, death (Samreen, 2009). Often symptoms are not severe, or may be absent, and consequently hyperglycaemia sufficient to cause pathological and functional changes may be present for a long time before diagnosis is made. The long term effects of diabetes mellitus include progressive development of the specific complications of retinopathy with potential blindness, nephropathy that may lead to renal failure, and/or neuropathy with risks of foot ulcers, amputation, charcot joints, and features of autonomic dysfunction. People with diabetes are at increased risk of cardiovascular, peripheral vascular and cerebrovascular disease (WHO, 1999). No wonder American Stroke Association (2012) stated that at least 68% of people greater than 65 years of age with diabetes die of some form of heart disease while 16% die of stroke. It has also been noticed that hypertension is twice as frequent in patients with diabetes compared with patients without the disease.

**2.6.1 Types of diabetes**

There are several forms of diabetes. Scientists are still defining and categorizing some of these varieties and establishing their prevalence in the population (Samreen 2009). However, the majortypes of diabetes are type 1 DM, type 2 DM, Gestational Diabetes and secondary DM.

**Type 1 diabetes:**

It is an autoimmune disease in which the immune system mistakenly destroys the insulin-making beta cells of the pancreas. An individual with a type 1 process maybe metabolically normal before the disease is clinically manifest, but the process of beta-cell destruction can be detected. It is usually characterized by the presence of islet cell or insulin antibodies which identify the autoimmune processes that lead to beta-cell destruction (WHO, 1999). This form of diabetes is usually diagnosed in children and adolescents, and sometimes in young adults. To survive, patients must administer insulin medication regularly. Type 1 was formerly called insulin dependent DM and juvenile diabetes.

**Type 2 diabetes:**

It is a disorder of metabolism, usually involving excess weight and insulin resistance. In patients with this type of DM, the pancreas makes insulin initially, but the body has trouble using this glucose controlling hormone. Eventually the pancreas cannot produce enough insulin to respond to the body’s need for it (Samreen, 2009).

**Gestational diabetes:**

It is a temporary metabolic disorder that any previously non-diabetic woman can develop during pregnancy, usually the third trimester. Hormonal changes contribute to this disease, along with excess weight and family history of diabetes. Gestational diabetes can cause problems for the mother and baby including preeclampsia, premature delivery, oversized infant, jaundice and breathing difficulties in the infant (Samreen, 2009). This disease typically ends when the pregnancy does, but it increases the risk of type 2 diabetes later in life for the mother and the child (WHO, 1999).

**Secondary Diabetes:**

These are diabetes caused by another condition. The many potential sources of secondary diabetes range from diseases such as pancreatitis, cystic fibrosis, Down syndrome and hemochromatosis, to medical treatments including corticosteroids, immune suppressives, diuretics and pancreatectomy .

**2.6.2Risk factors and causes of diabetes**

Genetics and family history

Overweight and obesity

Other diseases like high blood pressure, hyperlipidemia, asthma and sleep apnea

Lack of physical activity

Improper diet

Hormones like cortisols have been linked to fluctuating glucose levels in type 2 Diabetes.

Drugs such as streptozotocin and alloxan can induce diabetes

**2.7 HYPOLIDIPDERMIA**

Hypolipidemia is a decrease in plasma lipoprotein caused by primary (genetic) or secondary factors. It is usually asymptomatic and diagnosed incidentally on routine lipid screening. Treatment of secondary hypolipidemia involves treating underlying disorders. Treatment of primary hypolipidemia is often unnecessary, but patients with some genetic disorders require high-dose vitamin E and dietary supplementation of fats and other fat-soluble vitamins.

**2.7.1 Lipid peroxidation** refers to the oxidative deterioration of lipid. It is the process in which free radicals ‘steal’ electrons from the lipids in cell membranes resulting in cell damage. Lipid peroxidation proceeds by free radical chain reaction. Polyunsaturated fatty acids are most often being affected because of the presence of multiple double bonds in between which lie methylene bridges (-CH2-) that possess reactive hydrogens.When the radical removes hydrogen atom, it leaves behind an unpaired electron in the lipid. This in turn leads to chain reaction.

L-H + OH .→ H2O + L .

The lipid radicals formed lead to cell damage. Three mechanisms are able to induce lipid peroxidation: autoxidation (by free radicals reaction), photoxidation and enzyme action.

Autoxidation is a radical-chain process involving three stages: initiation, propagation and termination. The general process of lipid peroxidation consists of three stages: Initiation, propagation and termination

**Initiation** occurs when oxygen is partly reduced by

Fe 2+ to NADPH-Oxidase SOD Myeloperoxidase

species able to abstract a hydrogen atom from a methylene carbon .The resulting alkyl radical reacts with oxygen to form a peroxy radical (LOO .), which itself can liberate LOOH via hydrogen abstraction from a neighbouring alkyl bonds.

**In propagation**, fatty acid radicals react with molecular oxygen forming a peroxyl-fatty acid radical. This radical is also an unstable species that reacts with another free radical acid, producing a different fatty radical and a lipid peroxide or acyclic peroxide if it had reacted with itself. The cycle continues as the new fatty acid radical react in the same way.

LOO.+ L H LOOH + L (propagation)

**Termination** occurs when new radicals reacts and produce a non-radical species. Anioxidant vitamin E and antioxidant enzymes play a major role in the termination process.

Photo-oxidation occurs when singlet oxygen of highly electrophilic reacts with unsaturated lipids. In the presence of sensitizers (chlorophyll, porphrins, myoglobin,riboflavin, bilirubin), a double bond interacts with singlet oxygen produced from O2 by light. The oxygen is added at either end carbon of a double bond which takes the trans-configuration. Thus, the possible reaction of singlet oxygen with double bond produces hydroperoxides .

**2.8 ANTIOXIDANTS**

Antioxidants are man-made or natural substances that may prevent or delay some types of cell damage. Antioxidants are found in many foods, including fruits and vegetables. They are also available as dietary supplements. Examples of antioxidants include;Beta-carotene , Lutein , Lycopene , Selenium , Vitamin A , Vitamin C , Vitamin E.

Vegetables and fruits are rich sources of antioxidants. There is good evidence that eating a diet with lots of vegetables and fruits is healthy and lowers risks of certain diseases. But it isn't clear whether this is because of the antioxidants, something else in the foods, or other factors.

High-dose supplements of antioxidants may be linked to health risks in some cases. For example, high doses of beta-carotene may increase the risk of lung cancer in smokers. High doses of vitamin E may increase risks of prostate cancer and one type of stroke. Antioxidant supplements may also interact with some medicines.

There are anti oxidant foods we can take but should be taken with caution and they are; Goji berries , Wild blueberries , Dark chocolate , Pecans , Elderberries , Kidney beans , Cranberries, Blackberries , Cilantro , Other high antioxidant foods not listed above, which are still great sources and highly beneficial, include common foods like tomatoes, carrots, pumpkin seeds, sweet potatoes, pomegranates, strawberries, kale, broccoli, grapes or red wine, squash, and wild-caught salmon. Try to consume at least three to four servings daily of these high antioxidant foods (even more is better) for optimal health.

**CHAPTER THREE**

**3.0 MATERIALS AND METHODS**

**3.1 CHEMICALS AND REAGENTS**

Methanol , Alloxan , Glibenclamide (5mg) , Distilled water , DMSO dimethylsulphuroxide , Normal saline , Lipid profile kit , Hydrogen peroxidase

**3.2 EQUIPMENT**

Glucometer , Weighing balance , Oven , Centrifuge , Spectrophotometer , water bath , electronic blender , beaker , stirrer , measuring cylinder.

**3.3 MATERIALS**

Glucose strips , Wahtsmann (no.1) filter paper , Filter cloth , Latex glove , Animal cages , Glass wares , Foil , Beaker , Spatula , Hand towel , Razor blade , Funnel , Beaker , 5ml and 2ml syringes , EDTA bottles

**3.4 THE PLANT**

The leaves of *kigelia Africana* were obtained from Omega , Ekwetekwe , Umuzeroko , Ebonyi state , Nigeria . They were authenticated by the botanist at the department of plants and environmental sciences , university of nsukka, Nigeria.

**3.4.1 EXTRACT PREPARATION**

The fresh leaves were shade dried for 3 days

They were ground to a powdered form .

The powdered leaves which was 500.82g were now soaked in 500ml of methanol for 3 days and stirred .

The extract was filtered with a filter cloth and then filtered under suction pressure with a wahtsmann filter paper.

All extracts were dried using rotory evaporator.

**3.4.2 PREPARATION OF EXTRACT FOR ADMINISTRATION**

A quantity of 4.9g of the dried filtrate was placed in a beaker and 1ml of DMSO was added and stirred and made to 50ml with normal saline.

**3.5 EXPERIMENTAL ANIMAL**

A total of twenty apparently healthy wister albino male rats (Rattus norvegicus) were obtained from the Department of Zoology, university of Nigeria , nsukka , Enugu state Nigeria . the animals were allowed to acclimatize for one week before commencement of experiment. They were allowed access to water and feed (Vital Agricultural feeds) , throughout the period of the experiment.

**3.5.1 ANIMAL SEPERATIONS , INDUCTION AND TREATMENT MEASURES**

**The induction** of alloxan was done intraperitoneally with 180mg/kg body weight of the alloxan and diabetes was confirmed after 72 hours.

Twenty wister albino male rats were assigned into 4 different groups which had five rats in each of the group

**GROUP 1;**Normal control was fed with feed and water

**GROUP 2;**Diabetic untreated was fed with feed and water

**GROUP 3;**Diabetic treated was fed with water , feed and treated with Glibenclamide

**GROUP 4;**Diabetic treated was fed with water , feed and methanol leaf extract of kigelia Africana.

Treatment was done orally for a period of 14 days (daily).

**3.6 DETERMINATION OF THE BIOCHEMICAL PARAMETERS.**

All the choosen biochemical parameters were assayedusing randox bio diagnostic kits and the procedures were strictly followed as outlined in the manual guide.

**3.6.1 DETERMINATION OF TOTAL PROTEIN CONCENTRATION**

Total protein concentration was determined using protein assay kits according to the method

of Lowry *et al*. (1951)

**Principle**

This method is based on the principle that cupric ions, in an alkaline medium, interact with

peptide bonds of proteins resulting in the formation of a coloured complex. Reagents contained in assay kits (Randox company, USA)

Contents

1. Biuret Reagent conce

Sodium hydroxide 100mmol/L

Na – K – tartrate 16 mmol/L

Potassium iodide 15 mmol/L

Cupric sulphate 6 mmol/L

2. Blank Reagent

Sodium hydroxide 100 mmol/L

Na – K – tartrate 16 mmol/L

3. Standard

Protein 60 g/L (6.0 g/dl)

**Procedure**

Distilled water (0.02 ml) was pipetted into reagent blank (B) test tube only. Standard solution

(0.02 ml) was added to another test tube labeled ST (standard) only. After which 0.02 ml of

the sera from the different rats were added to different test tubes labeled SA (sample) only.

Biuret reagent (1.0 ml) was added to all the three sets of test tubes. The content was mixed thoroughly and incubated for 30 minutes at 25OC (room temperature). Absorbance of the

Sample (Asample) and of the Standard (Astandard) against the reagent blank was read at a

wavelength of 530 nm.

The total Protein concentration was calculated as follows:

Total Protein Conc. = Conc. Standard A A Standard Sample ×

Where:

A sample = Absorbance of the Sample

A standard = Absorbance of the Standard

**3.6.2 LIPID PEROXIDATION (MALONDIALDEHYDE MDA)**

Lipid peroxidation was determined spectrophotometrically by measuring the level of the lipid peroxidation product , malondialdehyde (MDA) as described by wallin *et al.,* (1993)

**Principle**

Malondialdehyde (MDA) reacts with thiobarbituric acid to form a red or pink coloured complex which in acid solution , absorbs maximally@532nm .

MDA +2TBA

**Reagent preparation**

1. 1.0%TBA; 1g of TBA was dissolved in 0.3% Naoh.

2. 25% TCA; 25g of TCA was dissolved in 100ml of distilled water.

3. 0.3% NaOH ; 0.3g of NaOH was dissolved in 100ml of distilled water.

**Procedure**

Ten microlitre ofBHT reagent was added to 250 microlitre of TBA reagent into a clean test tube and mix

Then incubate for 60 minutes at 60 degrees centigrade

Add ten microlitre of the sample to the mixture , mix well and pour into a cuvette and place in a spectrophotometer and take the absorbance at 532nm.

**3.4.2 DETERMINATION OF GLUTATHIONE PEROXIDASE (GPX)**

**Principle**

Glutahtione peroxidase (GPx) catalyses the oxidation of glutathione (GSH) by cumene hydroperoxide in the presence of glutathione reductase (GR) and NADPH. The oxidized glutathione (GSSG) is immediately converted to the reduced form with a concomitant oxidation of NADPH to NADP+. The decrease in absorbance at 340 nm is measured.

**Procedure**

Heparinized whole blood was used. The blood sample (0.05 ml) was diluted with 2 ml of diluting agent and mixed thoroughly. Diluted sample (0.02 ml) was pipetted into a sample test (T) tube only. Distilled water (0.02 ml) was pipetted into the reagent blank (B) test tube only. One milliliter (1.0 ml) of the reagent (R1) was pipetted into the sample (T) and reagent blank (B), both were placed in water bath at 37oC. Exactly 0.04 ml of cumene hydroperoxide was pipetted into both the sample test and reagent blank. Immediately the initial absorbance of sample test (T) and reagent blank (B) were read after one minute and again after 1 and 2 minutes at a wavelength of 340 nm.The blank value was subtracted from the sample value.

These reagents were mixed immediately and absorbance read at 340 nm

**3.6.4DETERMIATION TRIACYLGLYCERIDES**

**Principle**

The triacylglycerol concentration was determined after enzymatic hydrolysis with lipases. The indicator is a quinoneimine formed from hydrogen peroxide, 4-aminophenazone and 4-chlorophenol under the catalytic influence of peroxidase.

**Procedure**

One hundred microllitre (100 µl) of the reagent 1 was pipetted into the reagent blank tube, standard tube and the sample tubes. In the standard test tube was added 10 µl of the standard (CAL) while 10 µl of the sample was pipetted into the sample tube mixed thoroughly and incubated for 10 minutes at 20-250c. Absorbance of the sample and the standard were measured against the reagent blank within 60 minutes at 546 nm .Triacyglycerol concentration in

mmol/L = 2.29 A A Standard Sample × ∆ ∆

Asample = Absorbance of sample

Astandard = Absorbance of standard

2.29 = constant.

**3.6.5 DETERMINATION OF THE GLUCOSE LEVEL**

This assay is done to know the blood glucose levelof the experimental rats. Accu check machine was used to determine the glucose level.

**Principle**

The method is based on the rection of glucose and oxygen in the prescence of oxidase to yield gluconic acid and hydrogen peroxide subsequently oxidizes. The dye in the reaction mediated by peroxidase producing a blue colored form of dyes .The intensity of blue color is proportional to the glucose concentration in the sample and it is measured and read by ONE TOUCH meter.

The one-touch gluconometer was essentially a reflectance meter, the amount of light reflected in the reagent area of the dextrostix measured in a readour meter scale was a measure of the concentration of glucose in blood.

**Procedure**

Code key was inserted into the accu check machine code slot.

The code matched the test strip.

Glucometer strip was inserted into accu check machine properly.

The rat was bled in the tail and blood droplet was dropped on the strip

The result was displayed after 5 seconds in mg/dl.

**CHAPTER FOUR**

**RESULTS**

***Figure 1***–The result obtained from the analysis as shown in fig. 1 indicated that there was a significant decrease (p<0.05) in the group four which received the plant extract compared to the normal in group one. Also, the level of triacylglycerides in the group treated with the standard drug showed no significant different in the level triacylglyceridescompared to the normal group, whereas the untreated groups showed a significant increase (p>0.05) elevated tryacylgricerides level in the untreated group.

**4.1 Figure 1:shows chart of TAG levels in the treated and Control groups.**

***Figure 2***–the obtained in this study showed a significant decrease (p<0.05) in the total protein level of the treated groups compared to the normal, whereas the untreated group showed a significant increase (p>0.05) level of total protein in the untreated group compared to the normal group.

**Figure 2:Chart of Total ProteinConcentration in the treated and Control groups.**

***Figure 3***–the results obtained in this study when compared to the normal control showed a significant decrease (p<0.05) in the group four treated with the extract. It also showed GPx level in the groups treated with the standard diabetic drug and the untreated group.

**Figure 3:Chart of GPx in the treated and Control groups.**

**Figure 4**. A significant increase was observed in diabetic rats with standarddrug glib**:** An increase (p<0.05) not too significant was observed in the test group 4 treated with the plant extract compared with other groups glibenclamide compared with the normal group 1.

**FIGURE 4:Chart showing MDA in alloxan induced diabetic rats**

**4.5 Percentage yield for extraction**

**SAMPLE EXTRACT WEIGHT (g) PERCENTAGEYIELD**

**(%)**

Leaves dried and ground 500.33

Sample 0.65

Methanol extract 3.26

**4.6 Readings of Glucose test** Normal range is 95-150mg/dl

GROUPS 0 DAYS 3 DAYS 7DAYS 14DAYS

(Mg/dl) (Mg/dl) (Mg/dl) (Mg/dl)

Normal/ 102 101 101 96 Control` 95 96 95 90 100 97 97 95

110 110 105 98

98 98 96 93

Mean (Ẍ) 101 100 99 94

Diabetes 109 193 181 106

Not treated 101 210 187 118

111 189 179 102

97 200 179 104

102 195 180 113

Mean (Ẍ) 104 197 181 109

Diabetes treated 95 185 176 129

Glibenclamide 104 204 180 156

102 190 179 130

110 211 181 143

99 197 180 140

Mean (Ẍ) 102 197 179 139

Diabetes treated 103 213 189 116

With leaf extract 110 210 187 99

96 189 180 114

105 196 177 98 104 206 189 120

Mean (Ẍ) 103 203 184 109

**CHAPTER FIVE**

**5.1 DISCUSSION**

The present research studied the hypolipidaemic and antioxidant potentials of methanol leaf extract of *Kigelia africana* in alloxan-induced diabetic albino rats. The results showed that triacylglycerol (TAG) was highest in the untreated diabetic rats. This possibly shows that the diabetic condition may have precipitated the lipidaemic condition which was higher than the normal control rats. Treatment of the diabetic rats with Glibenclamide lead to reduction of the TAG (1.26 ± 0.05), which was lower than the value in the normal control.

*K. africana* extracts lead to further reduction of TAG to 1.14 ± 0.18. This shows that the leaf extract of the plant possesses possible anti-lipidaemic properties which lead to the least triacylglycerol concentration among all the experimental groups. The results also showed that the leaf extract of *K. africana* possessed more antilipidaemic properties compared to the oral antidiabetic drug (Glibenclamide).

The pathway affected by the plant extract is the pancreatic function of the Islets of Langerhans which provides insulin for glucose uptake into the cells of the muscles and adipose tissues. Diabetic condition induced by alloxan lead to the absence of glucose in the cells due to lack of uptake by insulin, leading to increase in plasma glucose in the diabetic rats. The absence of insulin in the diabetic rats also lead to the mobilization of stored TAG from the adipose tissues for use as alternative source of energy in the absence of glucose in the cells, leading to increased TAG in the diabetic non-treated rats. The leaf extracts of *K. africana* affected the pancreas positively leading to production of insulin and uptake of glucose and also decrease in the mobilization of fat from the adipose tissue stores. This led to decreased plasma TAG as observed in the diabetic rats treated with the extract when compared with the other groups of animals.

The total protein assay sowed that the highest concentration was in the diabetic non-treated group (5.48 ± 0.89) followed by (4.44 ± 0.43) in Normal control group. The diabetic group treated with Glibenclamide had the least protein concentration (3.82 ± 0.29), while the diabetic rats treated with *K. africana* extract (3.88 ± 0.18). The decrease in protein concentration in the two treated groups may suggest recovery from the diabetic condition and any possible complication. The plant extract however was not able to reduce the protein concentration to the extent that Glibenclamide did.

The assay for glutathione peroxidase (GPx) in eth different groups of experimental animals showed that diabetic rats treated with Glybenclamide had the highest activity (39.65 ± 2.02), followed by the diabetic non-treated group (38.70 ± 3.70). The diabetic rats treated with the plant extract had 29.73 ± 4.87 while the least activity was observed in the Normal control (29.20 ± 0.72).

Gltathione peroxidase (GPx) is the most important enzyme responsible for detoxification of peroxides in living cells. It catalyzes the reduction of hydroperoxides, including hydrogen peroxide by reduced glutathione. Form the study, lipid metabolism in the diabetic rats possibly led to the production of free radicals and the need for increased action of GPx.

However although the leaf extract of *K. africana* led to the increased activity of GPx compared to the normal control rats, the GPx activity was not as increased as that observed in the diabetic rats treated with Glybenclamide and the diabetic untreated group. The administration of the plant extract affected the pathway of glutathione peroxidation whereby reduced glutathione is used to reduce hydrogen peroxide to water and hence protecting the integrity of cells from free radicals.

The assay for malondialdehyde (MDA) showed that the highest concentration of MDA was in the diabetic non-treated group, while the group treated with Glybenclamide a standard antidiabetic drugs showed a reduced level of MDA in the study group. Also, the diabetic group treated with the extract showed a slight reduction in the MDA concentration compared to the untreated.This antioxidant property is also essential to protect the cells from the effect of radicals, and the results showed that the treatment with the extract led to a decrease in MDA concentration, which was increased by the diabetic condition of the animals that were untreated. This shows that the extract had antioxidant properties as observed by the levels of MDA in the treated group, compared to the other groups.

**5.2 CONCLUSION:**

The study showed that the leaf extract had anti-lipidaemic properties as shown by decrease in TAG. The extract also had antioxidant properties by slight decrease in GPx and marked decrease in MDA in the experimental animals.

**5.3 RECOMMENDATION**

It is recommended that *K.africana* can be used in the management of diabetes and lipid disease conditions if properly assessed and confirmed not to have any adverse effect.

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**APPENDIX A**

**MEAN ± SD OF THE ASSAYED PARAMETERS IN THE TREATED AND CONTROL RATS AND THE COMPARISON BETWEEN THE GROUPS**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Parameters** | **Normal Control** | **Diabetic non-treated** | **Diabetic + Glibenclamide** | **Diabetic + *K. afriana*** | **ANOVA** | |
| **F-Value** | **P=Value** |
| **TAG** | 1.28 ± 0.13 | 1.34 ± 0.11 | 1.26 ± 0.05 | 1.14 ± 0.18 | 2.131 | 0.136 |
| **Total Protein** | 4.44 ± 0.43 | 5.48 ± 0.89 | 3.82 ± 0.29 | 3.88 ± 0.18 | 10.766 | 0.000 |
| **GPx** | 29.20 ± 0.72 | 38.70 ± 3.70 | 39.65 ± 2.02 | 29.73 ± 4.87 | 15.049 | 0.000 |
| **MDA** | 2.49 ± 0.28 | 2.25 ± 0.99 | 2.71 ± 0.75 | 2.62 ± 0.65 | 0.390 | 0.762 |

**APPENDIX B**

**PERCENTAGE YIELD FOR THE EXTRACT**

**WEIGHT OF DRIED SAMPLE = 500.23**

**WEIGHT OF DRIED METHANOL EXTRACT = 3.26**

**% yield = 3.26 x 100 / 500.33**

**=0.65**

**APPENDIX C**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Descriptives** | | | | | | | | | |
|  | | N | Mean | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | | Minimum | Maximum |
| Lower Bound | Upper Bound |
| TAG | Normal Ctrl | 5 | 1.2800 | .13038 | .05831 | 1.1181 | 1.4419 | 1.20 | 1.50 |
| Diabetic (not treated) | 5 | 1.3400 | .11402 | .05099 | 1.1984 | 1.4816 | 1.20 | 1.50 |
| Diabetic+Glibenclamide | 5 | 1.2600 | .05477 | .02449 | 1.1920 | 1.3280 | 1.20 | 1.30 |
| Diabetic + K. africana | 5 | 1.1400 | .18166 | .08124 | .9144 | 1.3656 | .90 | 1.40 |
| Total | 20 | 1.2550 | .13945 | .03118 | 1.1897 | 1.3203 | .90 | 1.50 |
| Total\_protein | Normal Ctrl | 5 | 4.4400 | .43359 | .19391 | 3.9016 | 4.9784 | 3.90 | 4.90 |
| Diabetic (not treated) | 5 | 5.4800 | .88994 | .39799 | 4.3750 | 6.5850 | 4.40 | 6.50 |
| Diabetic+Glibenclamide | 5 | 3.8200 | .29496 | .13191 | 3.4538 | 4.1862 | 3.30 | 4.00 |
| Diabetic + K. africana | 5 | 3.8800 | .17889 | .08000 | 3.6579 | 4.1021 | 3.70 | 4.10 |
| Total | 20 | 4.4050 | .83570 | .18687 | 4.0139 | 4.7961 | 3.30 | 6.50 |
| GPx | Normal Ctrl | 5 | 29.1960 | .72061 | .32227 | 28.3012 | 30.0908 | 28.68 | 30.40 |
| Diabetic (not treated) | 5 | 38.6960 | 3.70206 | 1.65561 | 34.0993 | 43.2927 | 33.72 | 42.93 |
| Diabetic+Glibenclamide | 5 | 39.6520 | 2.02711 | .90655 | 37.1350 | 42.1690 | 38.24 | 43.11 |
| Diabetic + K. africana | 5 | 29.7320 | 4.86824 | 2.17714 | 23.6873 | 35.7767 | 27.20 | 38.42 |
| Total | 20 | 34.3190 | 5.81539 | 1.30036 | 31.5973 | 37.0407 | 27.20 | 43.11 |
| MDA | Normal Ctrl | 5 | 2.4920 | .28438 | .12718 | 2.1389 | 2.8451 | 2.12 | 2.87 |
| Diabetic (not treated) | 5 | 2.2520 | .99470 | .44484 | 1.0169 | 3.4871 | 1.11 | 3.43 |
| Diabetic+Glibenclamide | 5 | 2.7140 | .75215 | .33637 | 1.7801 | 3.6479 | 1.38 | 3.21 |
| Diabetic + K. africana | 5 | 2.6220 | .65354 | .29227 | 1.8105 | 3.4335 | 1.85 | 3.61 |
| Total | 20 | 2.5200 | .68271 | .15266 | 2.2005 | 2.8395 | 1.11 | 3.61 |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **ANOVA** | | | | | | |
|  | | Sum of Squares | df | Mean Square | F | Sig. |
| TAG | Between Groups | .105 | 3 | .035 | 2.131 | .136 |
| Within Groups | .264 | 16 | .016 |  |  |
| Total | .369 | 19 |  |  |  |
| Total\_protein | Between Groups | 8.874 | 3 | 2.958 | 10.766 | .000 |
| Within Groups | 4.396 | 16 | .275 |  |  |
| Total | 13.270 | 19 |  |  |  |
| GPx | Between Groups | 474.424 | 3 | 158.141 | 15.049 | .000 |
| Within Groups | 168.134 | 16 | 10.508 |  |  |
| Total | 642.557 | 19 |  |  |  |
| MDA | Between Groups | .603 | 3 | .201 | .390 | .762 |
| Within Groups | 8.253 | 16 | .516 |  |  |
| Total | 8.856 | 19 |  |  |  |

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Multiple Comparisons** | | | | | | | |
| LSD | | | | | | | |
| Dependent Variable | (I) BIOCHEMICAL\_PARAMETERS | (J) BIOCHEMICAL\_PARAMETERS | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
| Lower Bound | Upper Bound |
| TAG | Normal Ctrl | Diabetic (not treated) | -.06000 | .08124 | .471 | -.2322 | .1122 |
| Diabetic+Glibenclamide | .02000 | .08124 | .809 | -.1522 | .1922 |
| Diabetic + K. africana | .14000 | .08124 | .104 | -.0322 | .3122 |
| Diabetic (not treated) | Normal Ctrl | .06000 | .08124 | .471 | -.1122 | .2322 |
| Diabetic+Glibenclamide | .08000 | .08124 | .339 | -.0922 | .2522 |
| Diabetic + K. africana | .20000\* | .08124 | .026 | .0278 | .3722 |
| Diabetic+Glibenclamide | Normal Ctrl | -.02000 | .08124 | .809 | -.1922 | .1522 |
| Diabetic (not treated) | -.08000 | .08124 | .339 | -.2522 | .0922 |
| Diabetic + K. africana | .12000 | .08124 | .159 | -.0522 | .2922 |
| Diabetic + K. africana | Normal Ctrl | -.14000 | .08124 | .104 | -.3122 | .0322 |
| Diabetic (not treated) | -.20000\* | .08124 | .026 | -.3722 | -.0278 |
| Diabetic+Glibenclamide | -.12000 | .08124 | .159 | -.2922 | .0522 |
| Total\_protein | Normal Ctrl | Diabetic (not treated) | -1.04000\* | .33151 | .006 | -1.7428 | -.3372 |
| Diabetic+Glibenclamide | .62000 | .33151 | .080 | -.0828 | 1.3228 |
| Diabetic + K. africana | .56000 | .33151 | .111 | -.1428 | 1.2628 |
| Diabetic (not treated) | Normal Ctrl | 1.04000\* | .33151 | .006 | .3372 | 1.7428 |
| Diabetic+Glibenclamide | 1.66000\* | .33151 | .000 | .9572 | 2.3628 |
| Diabetic + K. africana | 1.60000\* | .33151 | .000 | .8972 | 2.3028 |
| Diabetic+Glibenclamide | Normal Ctrl | -.62000 | .33151 | .080 | -1.3228 | .0828 |
| Diabetic (not treated) | -1.66000\* | .33151 | .000 | -2.3628 | -.9572 |
| Diabetic + K. africana | -.06000 | .33151 | .859 | -.7628 | .6428 |
| Diabetic + K. africana | Normal Ctrl | -.56000 | .33151 | .111 | -1.2628 | .1428 |
| Diabetic (not treated) | -1.60000\* | .33151 | .000 | -2.3028 | -.8972 |
| Diabetic+Glibenclamide | .06000 | .33151 | .859 | -.6428 | .7628 |
| GPx | Normal Ctrl | Diabetic (not treated) | -9.50000\* | 2.05020 | .000 | -13.8462 | -5.1538 |
| Diabetic+Glibenclamide | -10.45600\* | 2.05020 | .000 | -14.8022 | -6.1098 |
| Diabetic + K. africana | -.53600 | 2.05020 | .797 | -4.8822 | 3.8102 |
| Diabetic (not treated) | Normal Ctrl | 9.50000\* | 2.05020 | .000 | 5.1538 | 13.8462 |
| Diabetic+Glibenclamide | -.95600 | 2.05020 | .647 | -5.3022 | 3.3902 |
| Diabetic + K. africana | 8.96400\* | 2.05020 | .000 | 4.6178 | 13.3102 |
| Diabetic+Glibenclamide | Normal Ctrl | 10.45600\* | 2.05020 | .000 | 6.1098 | 14.8022 |
| Diabetic (not treated) | .95600 | 2.05020 | .647 | -3.3902 | 5.3022 |
| Diabetic + K. africana | 9.92000\* | 2.05020 | .000 | 5.5738 | 14.2662 |
| Diabetic + K. africana | Normal Ctrl | .53600 | 2.05020 | .797 | -3.8102 | 4.8822 |
| Diabetic (not treated) | -8.96400\* | 2.05020 | .000 | -13.3102 | -4.6178 |
| Diabetic+Glibenclamide | -9.92000\* | 2.05020 | .000 | -14.2662 | -5.5738 |
| MDA | Normal Ctrl | Diabetic (not treated) | .24000 | .45422 | .604 | -.7229 | 1.2029 |
| Diabetic+Glibenclamide | -.22200 | .45422 | .632 | -1.1849 | .7409 |
| Diabetic + K. africana | -.13000 | .45422 | .778 | -1.0929 | .8329 |
| Diabetic (not treated) | Normal Ctrl | -.24000 | .45422 | .604 | -1.2029 | .7229 |
| Diabetic+Glibenclamide | -.46200 | .45422 | .324 | -1.4249 | .5009 |
| Diabetic + K. africana | -.37000 | .45422 | .427 | -1.3329 | .5929 |
| Diabetic+Glibenclamide | Normal Ctrl | .22200 | .45422 | .632 | -.7409 | 1.1849 |
| Diabetic (not treated) | .46200 | .45422 | .324 | -.5009 | 1.4249 |
| Diabetic + K. africana | .09200 | .45422 | .842 | -.8709 | 1.0549 |
| Diabetic + K. africana | Normal Ctrl | .13000 | .45422 | .778 | -.8329 | 1.0929 |
| Diabetic (not treated) | .37000 | .45422 | .427 | -.5929 | 1.3329 |
| Diabetic+Glibenclamide | -.09200 | .45422 | .842 | -1.0549 | .8709 |
| \*. The mean difference is significant at the 0.05 level. | | | | | | | |